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Synthesis of Fatty Acids by Yeast Particles

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ABSTRACT

KLEIN, HAROLD P. (Ames Research Center, Moffett Field, Calif.). Synthesis of fatty acids by yeast particles. J. Bacteriol. **92**:130–135. 1966.—When a mitochondria-free homogenate of *Saccharomyces cerevisiae* was centrifuged at 100,000 × *g* for 60 min, the sedimented crude particles incorporated acetate into fatty acids, but not into nonsaponifiable lipids. Degradation of the fatty acids formed indicated this to be de novo synthesis rather than chain elongation. Subfractions of the crude particles were obtained. The "ribosomal" fraction was unable to synthesize fatty acids, but had properties indicating the presence of acetokinase, fatty acid desaturase, and, probably, acetyl-coenzyme A carboxylase. A "light" particle fraction with a high specific activity of fatty acid synthetase was also obtained. Fatty acid synthesis by the "soluble" supernatant fluid appeared to be the result of contamination by the "light" particles. The data suggested the presence of several particulate entities in mitochondria-free homogenates.

Several years ago, we reported (1) that particles sedimented at 100,000 × *g* from a mitochondria-free homogenate of *Saccharomyces cerevisiae* were able to incorporate acetate, acetyl-coenzyme A (acetyl-CoA), or malonyl-coenzyme A (malonyl-CoA) into long-chain fatty acids. Later, it was reported (5) that this crude particle fraction could be further fractionated into three subfractions: a fluffy layer, a reddish layer, and a clear layer, and that, of these, only the reddish layer could stimulate the incorporation of acetate into fatty acids and into sterols in the presence of soluble supernatant fluid (6). This subfraction was indistinguishable from ribosomes by electron microscopy, ultracentrifugal analysis, and chemical composition (5, 6), and will be referred to here as the "ribosomal" fraction.

As will be seen in the present report, the ribosomal fraction does not approach the activity of the crude particles in incorporating acetate into fatty acids in the absence of soluble supernatant fluid. The studies to be detailed here indicate that the different enzymatic activities involved in formation of fatty acid from acetate by crude particles become separated by the subfractionation procedures.

MATERIALS AND METHODS

The organism used in this study was *S. cerevisiae* strain LK2G12. Earlier publications contain information pertinent to the methods used for cultivating the organism, preparing cell-free homogenates, and isolating the lipid fractions (4, 6). Other publications give details concerning fractionation of the crude, small-particle material (5, 6), methods used in the collection and separation of fatty acids by gas chromatography, thin-layer chromatography, and radioactivity determinations (4, 11). For the decarboxylation of radioactive fatty acids, a modification of the method of Schmidt (9) was used.

RESULTS

Fatty acid synthesis of crude-particle preparations. Table 1 shows the activity of the crude particles in incorporating acetate into lipids. It can be seen that, unlike the crude homogenate or the soluble supernatant fluid, the particle preparation incorporated acetate almost exclusively into the fatty-acid fraction. It should be further noted that this fraction, on a protein basis, was more active than the soluble supernatant fluid. Under these conditions, crude particle preparations routinely had a higher specific activity than the soluble supernatant fluid (see also Table 5). As is seen in Table 2, the crude particles incorporated acetate into several fatty acids. Furthermore, the products contained unsaturated acids in preference to saturated acids. This was in contrast to the activity of the soluble supernatant fraction, which generally yielded higher amounts of saturated fatty acids than unsaturated fatty acids. It is also interesting to note that preparations con-

TABLE 1. *Incorporation of acetate into lipids by supernatant fluid and particle fractions*[a]

Fraction used	Counts per min per mg of protein		FAF/NSF
	FAF[b]	NSF[b]	
Crude homogenate	20,600	4,830	4.3
Soluble supernatant fluid	11,600	2,630	4.4
Particles	19,000	210	90.6

[a] All tubes contained (in micromoles): sodium acetate, 20 (1.7×10^7 count/min); adenosine triphosphate (ATP), 100; nicotinamide adenine dinucleotide phosphate (NADP), 5; CoA, 1; isocitrate, 50; isocitric dehydrogenase, 500 units; $MgCl_2$, 10; and $MnCl_2$, 30; in a total volume of 12.4 ml. The fractions were made up in 0.1 M phosphate buffer (*p*H 7); crude homogenate contained 135 mg of protein; soluble supernatant fluid, 105 mg of protein; particles, 38 mg of protein. Incubation was for 1 hr at 30 C in air.

[b] FAF, fatty acid fraction; NSF, nonsaponifiable fraction.

taining particles generally incorporated acetate into C14 and C16 acids, whereas supernatant preparations yielded C16 and C18 acids as the main products. This situation is not clearly understood. It was not seen with malonyl-CoA as the substrate (e.g., Table 6), and may have been a reflection of the relative concentrations of acetyl-CoA carboxylase in the two types of preparations (3).

Since the crude particle fraction apparently converted acetate into long-chain fatty acids, it seemed reasonable that this particle fraction contained the enzymes acetokinase, acetyl-CoA carboxylase, fatty acid synthetase, and fatty acid desaturase. Further evidence for the presence of acetokinase and acetyl-CoA carboxylase is given in Table 3, from which it is seen that, in the presence of suitable cofactors, the omission of bicarbonate or of CoA drastically reduced the ability of this preparation to incorporate acetate into fatty acids. The effects noted in this table on the small amounts of nonsaponifiable lipids are probably not significant.

That the crude particles were, indeed, carrying out a de novo synthesis of fatty acids, and not just simple chain elongation of pre-existing fatty acids, was already suggested by the analysis of radioactive fatty acids formed, by use of gas chromatography. Since the main endogenous fatty acids are C16 and C18, simple chain elongation should be expected to yield C18 and C20 labeled acids from acetate. However, the main fatty-acid products from acetate were C14 and C16 fatty acids (Table 2). More rigorous proof of de novo synthesis was obtained by isolating individual radioactive fatty acids and then subjecting these to a Schmidt degradation. In this procedure, the terminal carbon of the acid was converted to carbon dioxide, which was trapped and counted. This count was compared with that of the original fatty acid. As is seen in Table 4, the degradation data supported the contention

TABLE 2. *Acetate incorporation into individual fatty acids*[a]

Fatty acid[b]	Percentage of total incorporation		
	Soluble supernatant fluid	Soluble supernatant fluid plus crude particles	Crude particles
<C12	1.0	9.0	7.8
C12	1.4	9.5	11.3
C12 to C14	1.0	2.5	3.2
C14	3.4	6.0	5.4
C14:1	0.7	20.0	24.4
C14:1 to C16	—	3.6	5.9
C16	25.0	7.1	6.6
C16:1	22.0	29.0	23.2
C16:1 to C18	1.7	2.7	6.0
C18	12.0	6.4	1.5
C18:1	28.0	3.0	2.8
>C18:1	5.0	1.8	2.0

[a] Soluble supernatant fluid (54 mg of protein) or crude particles (40.8 mg of protein), or both, were incubated, as indicated, with acetate-*1*-C^{14} (12 μM) and cofactors, in a total volume of 6 ml at 30 C for 1 hr in air. Fatty acids were extracted, methylated, and chromatographed. Recovery of counts added to columns was 66% in the case of crude particles, and over 85% in other cases.

[b] Figures refer to chain length and degree of unsaturation.

TABLE 3. *Dependence of fatty acid synthesis on CO_2 and CoA, using crude small-particle preparations*[a]

Ingredient omitted	Expt 1		Expt 2	
	FAF	NSF	FAF	NSF
None	19,790[b]	1,670	46,200	560
$KHCO_3$	730	0	—	—
CoA	—	—	4,270	380

[a] Crude particles (7.0 mg of protein) were incubated with acetate-*1*-C^{14} (2 μM, 1.8×10^6 count/min), ATP (5 μM), NADP (0.5 μM), isocitrate (5.5 μM), isocitric dehydrogenase (50 units), potassium phosphate (100 μM, *p*H 7.3), CoA (0.1 μM), $KHCO_3$ (60 μM), and $MnCl_2$ (3 μM), in a total volume of 1.2 ml for 2 hr at 30 C in the air. NSF and FAF as defined in Table 1; — indicates not done.

[b] Results expressed as counts per minute.

that de novo synthesis is the major route of fatty acid formation by the crude particles.

Formation of fatty acid using fractionated particle preparations. When the individual subfractions were tested for their ability to incorporate acetate into fatty acids in the absence of soluble supernatant fluid, all of the preparations were found to be considerably less active on a protein basis than the crude particle preparations. However, the ribosomal fraction was just as active as the crude particles in the presence of soluble supernatant fluid in the incorporation of acetate into both fatty acids and nonsaponifiable lipids (Table 5). It is also to be noted (Table 6) that the ribosomal fraction yielded the identical pattern of fatty acids as the crude particles in the presence of supernatant fluid. As shown in Table 6, supernatant fluid alone yielded mainly saturated C16 and C18 acids from malonyl-CoA, and the addition of either particle preparation changed the pattern to the corresponding monounsaturated acids.

Since the ribosomal fraction actively stimulated the incorporation of acetate into lipids in the presence of soluble supernatant fluid, but was unable itself to carry out such an incorporation, it seemed likely that this particle fraction stimulated the supernatant fluid by supplying acetokinase and, possibly, acetyl-CoA carboxylase. However, this fraction did not appear to contain much fatty acid synthetase. Furthermore, since the latter enzyme was present in the crude particle preparations, it followed that the synthetase was destroyed during the fractionation, or that it became separated from the ribosomal fraction during subfractionation. It therefore seemed appropriate to test each of the three subfractions directly for fatty acid synthetase, by use of labeled malonyl-CoA. In these experiments, no synthetase activity was ever found in the clear layer. The ribosomal fraction was feebly active (Table 7), but the subfraction with the highest specific activity was the fluffy layer. Indeed, the

TABLE 4. *Decarboxylation of fatty acids*[a]

Fatty acid	Soluble supernatant	Crude particles	Soluble supernatant plus crude particles
Palmitic	1:6.9 1:6.8	1:5.6 1:6.2	1:6.1
Stearic	1:9.5 1:8.7	1:8.0 1:7.9	1:9.1

[a] After incubation with malonyl-CoA-*1,3*-C^{14}, fatty acids were extracted, methylated, and chromatographed. Palmitoleic and oleic esters were recovered, hydrolyzed, reduced to the corresponding saturated fatty acids, and then degraded. Duplicate determinations were made, except in the case of supernatant fluid plus particles. Theoretical ratios for de novo synthesis: palmitic, 1:8; stearic, 1:9; for simple chain elongation, 1:1 in each case. Results expressed as the ratio of labeled C1 to total C label.

TABLE 5. *Comparison of crude small particles with the "ribosomal" fraction in the incorporation of acetate into lipids in the presence and absence of soluble supernatant fluid*[a]

Soluble supernatant fluid (mg of protein used)	Crude particles (mg of protein used)	"Ribosomal" fraction (mg of protein used)	FAF (count/min incorporated)	NSF (count/min incorporated)
—	3.2	—	13,000	1,160
—	—	4.0	1,020	630
9.0	3.2	—	31,050	127,880
9.0	—	4.0	30,935	124,840
9.0	—	—	11,750	25,520

[a] In addition to preparations, all tubes contained, on a millimicromolar basis: NADP, 0.5; CoA, 0.1; ATP, 5; isocitrate, 5; isocitric dehydrogenase, 50 units; $MgCl_2$, 1; $KHCO_3$, 30; glutathione, 20; and acetate, 2 (7.5×10^5 count/min). Incubation was at 30 C for 1 hr in air. NSF and FAF as defined in Table 1.

TABLE 6. *Incorporation of malonyl-CoA into individual fatty acids*[a]

Fatty acid	Percentage of total incorporation		
	Soluble supernatant fluid	Soluble supernatant fluid plus crude particles	Soluble supernatant fluid plus ribosomal fraction
<C12	2.7	1.8	1.5
C12	2.6	1.7	1.5
C12 to C14	0.2	0.3	0.3
C14	3.4	1.2	1.0
C14:1	0.5	3.4	3.9
C16	31.0	2.1	1.9
C16:1	6.9	36.9	37.1
C16:1 to C18	1.2	4.3	3.5
C18	30.0	2.5	2.2
C18:1	15.8	37.4	38.6
>C18:1	5.6	8.1	8.4

[a] Soluble supernatant fluid (3.0 mg of protein) alone, or with crude particles (4.45 mg of protein) or ribosomal fraction (4.15 mg of protein), was incubated with malonyl-CoA-*1,3*-C^{14} (0.5 μM), acetyl-CoA (0.1 μM), and cofactors at 30 C for 20 min in air. Fatty acids were extracted, methylated, and chromatographed. Recovery of counts added to columns was greater than 80% in each case.

TABLE 7. *Incorporation of malonyl-CoA into fatty acids by soluble and particle preparations*[a]

Prepn	Protein (mg)	Activity (count/min)	Protein (mg)	Activity (count/min)	Protein (mg)	Activity (count/min)
Soluble supernatant	0.13	20,765	0.033	3,245	0.008	610
Ribosomal layer	0.16	4,715	0.039	1,580	0.009	405
Fluffy layer	0.15	138,270	0.038	70,080	0.009	12,640

[a] All tubes contained, on a millimicromolar basis: malonyl-CoA-*1,3-C*^{14}, 0.2 (5.7 × 10^5 count/min); acetyl-CoA, 0.05; isocitrate, 5; isocitric dehydrogenase, 50 units; NADP, 0.5; phosphate, 100 (*p*H 7.3); $MnCl_2$, 5; glutathione, 20; $KHCO_3$, 60; and ATP, 5; in a total volume of 1.0 ml. Incubation at 30 C for 15 min in air.

synthetase activity of the light, fluffy layer (which sediments just below the soluble supernatant fluid) was so high that it seemed plausible that the fatty acid-synthesizing activity of the soluble supernatant fluid itself might be the result of remaining traces of these light particles. The latter, it was felt, might not have been completely sedimented by the customary procedure used to obtain soluble supernatant fluid, i.e., centrifugation for 60 min at 100,000 × *g*. To test this contention, a mitochondria-free homogenate was subjected to centrifugation at 37,500 rev/min in a Spinco model L centrifuge (rotor no. 30) for increasing periods of time. Samples of supernatant fluid were removed at various times and tested for their rate of incorporation of malonyl-CoA into fatty acids. Figure 1 illustrates the loss of fatty acid-synthesizing capacity of the supernatant fluid as a function of the time of centrifugation. In this experiment, assuming (according to the *Beckman Model E Analytical Ultracentrifuge Instruction Manual*, p. 10–12) a solvent viscosity of 0.01 poise, a partial specific volume of the solvated particles of 0.74, and a solvent density of 1.0, one can calculate a diameter for the sedimenting rate-limiting particle for fatty acid synthesis. These calculations yielded a particle diameter of approximately 150 A, or a Svedberg constant of 45.

The fluffy layer also appeared to be virtually free from the desaturating system. In this experiment, the crude particles suspended in tris-(hydroxymethyl)aminomethane (Tris) buffer were layered on 10% sucrose in a centrifuge tube and then centrifuged, a procedure that effectively separated the fluffy layer (light particles) from the other fractions. The light particles remained suspended above the sucrose layer, whereas the heavy particles sedimented at the bottom of the tube. The light particles and the heavy particles were then compared with the crude particles for incorporation of malonyl-CoA into individual fatty acids. Once again, it is seen (Table 8) that the light particles were very active in fatty acid-synthesizing activity compared with the heavy-particle material. The latter still had some activity, presumably because of contamination by the lighter particles. What is important in the present context, however, is the fact that the desaturating activity appeared to sediment with the heavier particle fraction (see also Table 6).

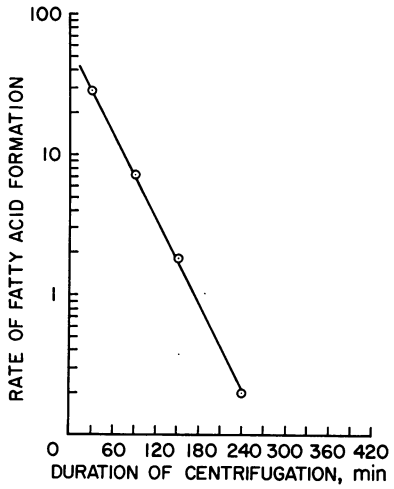

FIG. 1. *Fatty acid formation as a function of centrifugation. Millimicromoles of malonyl-CoA incorporated per minute by "soluble" supernatant fluid obtained after various periods of centrifugation.*

DISCUSSION

The crude, small-particle fraction of *S. cerevisiae*, obtained after removal of mitochondrial material by sedimentation at 100,000 × *g* for 60 min, contains the necessary enzymatic equipment to convert acetate to both saturated and unsaturated fatty acids. As has been pointed out before (6), this fraction also contains the system for cyclizing squalene to sterols. Upon subfractionation, the ribosomal fraction stimulates the synthesis of both fatty acids and nonsaponifiable materials in the presence of soluble supernatant fluid, even though these particles have little lipogenic activity alone. From the data presented

TABLE 8. *Separation of synthetase and desaturase activities during fatty acid synthesis*[a]

Individual acids	Total fatty acids		
	Crude particles (62,540 count/min)[b]	Light particles (410,235 count/min)[b]	Heavy particles (11,668 count/min)[b]
<C12	1.9[c]	1.0	1.8
C12	0.4	0.5	4.9
C12 to C14	—	0.1	0.3
C14	0.6	1.7	2.0
C14:1	6.1	2.7	4.6
C16	10.2	30.9	17.9
C16:1	35.9	3.6	26.8
C16:1 to C18	3.7	1.4	2.8
C18	13.2	44.3	15.6
C18:1	28.8	9.6	18.7
>C18:1	5.5	4.3	4.6

[a] Crude particles (247 mg of protein) were suspended in 13 ml of Tris (0.002 M, *p*H 6.8)-$MgCl_2$ (0.002 M), layered over 10% sucrose, then centrifuged at 30,000 rev/min for 65 min, by use of a no. 30 Spinco rotor. Top layer, containing light particles, was removed and centrifuged at 40,000 rev/min for 60 min, after which the pellet was resuspended in 1.0 ml of Tris-Mg buffer. The heavy-particle pellet was resuspended in 5 ml, same buffer. Particle samples were incubated with malonyl-CoA-*1,3*-C^{14} (0.1 μM, 5.5 $\times$ 10^5 count/min), acetyl-CoA (0.05 μM), and cofactors for 15 min at 30 C in air.

[b] Counts per minute per milligram of protein.

[c] Percentage of total.

here, it appears that this fraction may be so rich in the enzymes acetokinase and acetyl-CoA carboxylase that both fatty acid and nonsaponifiable-lipid synthesis may be stimulated when this fraction is added to the supernatant fluid. In this connection, we reported earlier (3) on the fact that the crude small particles had a high specific activity of acetyl-CoA carboxylase. The present data suggest that the ribosomal fraction must also contain the desaturating system. On the other hand, this fraction is deficient in fatty acid synthetase.

The light particles (obtained most readily by the use of a 10% sucrose medium) are particularly rich in the fatty acid synthetase. Since they do not incorporate acetate into fatty acids, and because the main fatty acid products from malonyl-CoA are saturated, the light particles appear to be deficient in the enzymes presumed to be present in the ribosomal fraction. Furthermore, the data of Fig. 1 strongly suggest that the rate-limiting enzyme involved in the formation of fatty acids from malonyl-CoA by mitochondria-free homogenates is a 45*S* particle, or that the activity is bound to such a particle. These findings thus support the observations of Lynen (7, 8), who reported purifying a 43*S* particle from yeast preparations by classical protein fractionation procedures. This particle, having a molecular weight of about 2.3 $\times$ 10^6, was claimed to be a multienzyme complex capable of carrying out the synthesis of long-chain fatty acids from malonyl-CoA. Thus, it would appear that the fatty-acid synthetase particle described by Lynen can be obtained directly by differential centrifugation from yeast homogenates. Furthermore, the present data would support the idea that the enzyme exists as a large complex in situ.

The question arises whether the various activities associated with the ribosomal fraction may not be simply ribosome-bound proteins. The data available on this point, although far from complete, do not support this contention. Various treatments known to destroy ribosomal structure, such as treatment of the particles with ethylenediaminetetraacetate (EDTA), deoxycholate, or ribonuclease, while releasing most of the bound ribonucleic acid of the preparation, do not solubilize the enzymatic activities under consideration here (6). After treatment with one of the agents mentioned, followed by centrifugation, the enzymatic activities still sediment as particles. At present, therefore, we consider that these enzymes exist as particles, and that they contaminate the ribosomal fraction. It is also possible, of course, that these particles may be loosely bound in some way to the ribosomes.

Similarly, the fatty acid synthetase particle sediments with a fraction that is relatively rich in lipids (5), suggesting a membrane-rich fraction. However, in this case also, this situation may be simply coincidental. Treatment of the light-particle material with deoxycholate or with deoxycholate plus lubrol (2, 10) was found to solubilize completely such "membrane" components as ergosterol and lecithin (*unpublished data*). However, even after these lipids had been solubilized, the fatty acid synthetase activity behaved as a particle upon centrifugation.

Certainly the evidence presented here is merely suggestive. But, on the basis of the data, we would suggest that several kinds of particles, in addition to ribosomes, may be present in the postmitochondrial suspensions obtained from yeast homogenates.

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